Manuscript title: Salivary IgA as a predictor of upper respiratory tract infections and relationship to training load in elite Rugby Union players

Brief running head: sIgA as a predictor of URTI in elite Rugby Union

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Salivary IgA as a predictor of Upper Respiratory Tract Infections and relationship to training load in elite Rugby Union players

ABSTRACT

Upper Respiratory Tract Infections (URTI) are amongst the most common illnesses reported in athletes. An URTI can result in missed training days, which in turn may lead to performance decrements. The purpose of this study was to investigate the use of salivary Immunoglobulin A (sIgA) as a predictor of URTI whilst also exploring the relationship to weekly training load in elite Rugby Union players.

Nineteen male elite Rugby Union players provided morning saliva swabs, bi-weekly (Monday and Friday), over a 10-week training period. Participants completed an illness log documenting symptoms of URTI. Session Rate of Perceived Exertion (sRPE) was collected to determine training load (sRPE x session duration). Weekly training load was also calculated. Logistic regression was used to analyze the relationship between incidences of URTI with sIgA and training load. Multi-level regression was conducted to compare associations between sIgA and training load.

The results found that the likelihood of suffering from an URTI increased when sIgA significantly decreased ($p=0.046$). Where sIgA decreased by 65% or more, a player was at a greater risk of contracting an URTI within the following 2-weeks. No association was found between sIgA and training load.

In conclusion, sIgA may be a useful predictor for determining the likelihood of players contracting an URTI. This will allow the coach to make informed decisions on training status, helping reduce the risk of players missing training, which may have performance decrements. Coaches will benefit from the fast, easy and instant results available, to analysis a player's immune function.

Key words:

Illness, Recovery, sIgA, Monitoring Markers, Performance

INTRODUCTION

For athletes to optimize performance, minimizing the number of training days missed due to injury and illness is essential. On average, athletes typically miss 15 training days per year and are unable to compete in at least 1 competition due to illness (39). Upper Respiratory Tract Infections (URTI) are one of the most common illnesses reported in athletes (17, 38). On average elite Rugby Union players experience 4 URTI over the course of a season (11-months) (7) and 89% of ice hockey players suffered from an URTI over a 6-month period (33). A number of studies have reported sIgA as a predictor of URTI in elite athletes (7, 27, 29, 33).

IgA is anti-body and an immune marker that is found in mucosal surface, including saliva (34). IgA is the first line of defense, it provides a barrier to viruses and antigens (22). sIgA binds to antigens and neutralizes viruses by inhibiting replication of pathogens (20), which can be responsible for causing URTI (9, 20, 22). Intensive training, and the associated decrease in secretion of sIgA, can lead athletes being more susceptible to URTI (27). A decrease in sIgA can increase the risk of contracting an URTI and interfere with training and thereby, increasing the chance of missing training days, which can lead to performance decrements (2, 31).

It has been found that lower sIgA levels significantly increased the risk of contracting an URTI in ice hockey players over a 6-month period (33) and soccer players over a 20-day period (27). Cunniffe et al. (7) studied elite Rugby Union players, observing that the players with lower mean sIgA reported higher incidences of upper respiratory infection (URI), however this was not found to be statistically significant. This may be due to the large

individual variability or infrequent testing of saliva, as swabs were only collected once a month, over an 11-month period. Similarly, Fahlman et al. (9) found mucosal IgA significantly decreased over periods of intense training, which significantly increased the incidence of URTI, in American football players. Tsai et al. (42) also found that a decrease in mucosal IgA significantly increased the risk of contracting an URTI, in taekwondo athletes. Interestingly, Fahlman et al. (9) and Gleeson et al. (13) both reported that lower IgA secretion rate may be used as a better predictor for URTI than IgA levels per se, as it actually represents the amount of IgA available in the mucosal surface. A limitation to both Fahlman et al. (9) and Orysiak et al. (33) studies were that saliva samples were only collected 8 time points over the course of the season. To date, just one longitudinal study, with the collection of weekly saliva samples has been conducted in elite professional sailors, over a 50-week period (29). This study found that if sIgA values decreased by 40%, 3-weeks later the athlete had a 50% chance of contracting an URTI (29). Consequently, in order to establish the relationship between sIgA and the incidence of URTI, further studies are required incorporating the frequent testing of saliva.

Research has found that high training loads can decrease sIgA (9, 25, 31) and suppress immune function, meaning individuals are more susceptible to suffering an URTI (34). Moreira et al. (25) found that URTI symptoms were more severe during periods of higher training. This study however, was only completed over 4-weeks of intensive futsal training. In contrast, it has been found that training load did not influence immune-endocrinal responses in judo athletes (1) or female basketball players (32). A limitation of these studies again was the infrequent collection of saliva samples, 6 times points over 19-weeks (1) and pre and post a 12-week training program (32). More frequent salivary monitoring is required to establish the weekly effect of training load on sIgA (7, 27, 40). Fahlman et al. (9) study

implied that the harder the training schedule, the greater reductions in sIgA and increased incidences of URTI, however the study did not quantify the intensity of training using any validated or reliable measure, it was only based on the training schedule. Similarly, Neville et al. (29) did not quantify training load. Instead, total hours of weekly training were calculated and separately, an index of sailing and training load were collected by a weekly scale, no daily internal measurement was taken to quantify training load, such as sRPE (18, 21). Further research is needed within elite team contact sports (7), with a reliable valid measure of internal training load (18, 21).

There are a considerable number of studies researching immune disturbance and training in elite athletes (7, 9, 14, 15, 25, 29). However, no study to date has been conducted with multiple testing time points during a week, in elite Rugby Union players. Additionally, there is limited research investigating the interaction between players' immune function (sIgA) and weekly training load (40). The aim of this study was to investigate the relationship between sIgA and URTI and whether sIgA could be used as a marker to predict URTI, in elite Rugby Union players over a 10-week training period. Secondly, this study aimed to investigate the effects of weekly training on immune function, while also exploring the relationship between sIgA, URTI and training load. It was hypothesized that (1) a decrease in sIgA would increase the risk of contracting an URTI and (2) there would be an inverse relationship between sIgA and training load.

METHODS

Experimental Approach to the Problem

Over a 10-week pre-season training period, players provided saliva samples twice a week, Monday and Friday morning, prior to training commencing. Monday saliva samples represented the start of the training week and Friday signified the last day of the training week. Prior to the Monday swab collection, players had at least one full day of recovery from training or playing a match. Players were required to complete a bi-weekly URTI log, at the same time as saliva samples were collected. The logs recorded any symptoms of URTI (e.g. cough, a runny nose, nasal congestion, headache, sore throat) (42). Players also could complete the log at any point throughout the week, the researcher, coaches or team doctor facilitated the log being provided. sRPE was taken after every session to calculate weekly training load. Baseline saliva data were collected after the players 3-week break from the previous season. The players had a download week in week 3 and matches were played in weeks 6-9 (Figure 1).

To ensure minimal disruption to training and continuity with the players' normal training schedule, all testing took place in the Rugby teams training facilities. The data collection for saliva and logging of an URTI took a maximum of 5-minutes to complete each morning.

Figure 1 here

Subjects

Nineteen elite male Rugby Union players volunteered to take part in the study (age 19.7 ± 1.1 years, height 184.5 ± 7.7 cm, body mass 96.2 ± 12.5 kg). All players were contracted for the

Academy of a professional Rugby team and trained full-time with the Academy and/or Senior team. All players had a minimum of 2-years training within an elite Rugby Academy and at least 2-years of Rugby playing experience before joining the Academy.

Typically, players trained 4-5 days a week, with multiple training sessions a day. These sessions included conditioning, gym/resistance and pitch based Rugby training. Prior to participating in the study, each player was screened and cleared of any signs or symptoms of illness and any allergies were noted. All players were informed of the study requirements (risks and benefits) and provided written informed consent. The study was approved by the University Research Ethics Committee.

Procedures

Saliva collection

All saliva samples were collected prior to any training and within 1-hour of the players wakening up. This was to limit the effect of diurnal variation (36). Throughout the 10-week training period, including baseline data collection, the method was standardized.

In week 1 of pre-season, baseline saliva samples were collected for 4-days (Monday, Tuesday, Thursday and Friday) in keeping with the player's normal training schedule. For the remaining pre-season testing, saliva samples were collected bi-weekly (Monday and Friday morning.

Players placed the oral fluid collector (OFC) swab (Soma Bioscience, Wallingford, UK) on top of their tongue and closed their mouth. They did not suck or move the swab around their mouth to ensure the test was consistent and reduced variability (6). The indicator on the stem

turned blue when the sample was complete; the swab collected 0.5ml of oral fluid. The swab was then placed in the OFC buffer bottle of assays (sodium phosphate, salts, detergents and preservatives), in line with the manufacturer's guidelines.

The researcher gently mixed the samples in the OFC buffer bottle for 2-minutes. Two drops of the sample were added to the sample window of the lateral flow device (LFD) and left for 15-minutes; this was the 'incubation' phase. The strip was placed in the Soma LFD real-time reader, with results ready within 22-seconds. The use of Soma Bioscience OFC collectors has been validated and proven to be a reliable method of collecting and analyzing salivary IgA compared to ELISA, (ICC $r = 0.89$, $p < 0.001$ and CV = 9.40%) (6).

Flow rate was calculated by dividing the total volume of saliva (0.5ml) by the time (minutes) it took the swab indicator to turn blue. Secretion rate was calculated by multiplying the sIgA concentration (ug.ml) by the saliva flow rate ($ml \cdot min^{-1}$) (9). Secretion rate is the total amount of sIgA present in the saliva surface per unit time (9) and flow rate is the amount of saliva produced by the salivary glands (19).

To reduce saliva measurement error and ensure more stringent testing (36), the players' were required to have consumed breakfast, refrained from brushing their teeth and eating chewing gum. In addition, players had to avoid drinking any caffeinated drinks (tea, coffee or sports drinks) or consuming alcohol 24-hours prior to testing (7). Each player recorded what they consumed for breakfast and completed a modified self-reported sleep diary (4, 41). Finally, any stressful situations the night before or that morning were also recorded, as research has found that stressful situations can affect saliva results (8). All nutrition was under the guidance of the team's nutritionist, players prepared their own snacks and pre-gym breakfast.

Upper respiratory tract infections (URTI)

A self-reported log was presented to the players on a Monday and Friday morning at the same time as the saliva swab collection, however players could complete the log at any point during the week, when symptoms arose. The log recorded symptoms, number of days with symptoms and severity of the infection (1-3); 1= no impact on daily activity, 2= infection had some impact on their daily activity, 3= infection led to a significant impact in daily activities (9, 24, 27). Verbal anchoring of the log was completed 2-days prior to testing and on the morning of the first testing day. Additionally, a 3rd anchoring day was completed 4-weeks into the study.

To be classified as an URTI, the infection had to last 2 or more days, and players had to present at least 2 of the following symptoms: cough, a runny nose, nasal congestion, headache, sore throat, sneezing, stuffy nose, nasal discharge, tight or wheezy chest, malaise and chilliness (42). If the player met the criteria of 2+ symptoms for 2+ days, the researcher followed up with the player to identify if it was an URTI, an illness, (e.g. fever, aches, and feeling sick) or an allergic reaction. If the researcher was unsure, the team doctor provided a diagnosis. This method of classifying URTI is consistent with previous research (9, 24, 27).

Training Load

To quantify the player's training session intensity, sRPE was recorded after every training session (21), using the modified Borg 0-10 scale (3). The players were asked individually 'how intense do you feel the session was?' (10) and reported their value to the coach or researcher to prevent any bias or influence from other players. Both gym and pitch sRPE were taken within 15-minutes of completing the session, as it has been found that sessions which do not finish at a high-intensity (related to gym sessions in this study) or include a cool down (pitch sessions in this study), do not affect the players perceived rating of the session (5). Compared to heart rate and blood concentrations, RPE has been found to be a valid and reliable monitoring marker for internal load and exercise intensity (21).

Training load for each session was calculated by sRPE x duration of session (minutes) (10). This calculation of training load has been found reliable and valid measure of training intensity (16). The sum of each sessions training load provided the quantification of weekly training load. Chronic load was calculated by averaging 4-weeks of training load data e.g. weeks 1-4, 2-5, 3-6 (11).

Statistical Analysis

Descriptive statistics were calculated for all variables and assumptions for parametric analysis were explored. Natural log transformation was used to calculate means, due to the variability in saliva (23). Significance levels were set at $p<0.05$ and highly significance was set at $p<0.001$.

All statistical analysis was conducted using MLwin software (version 2.36). Multi-level logistic regression was used for the URTI data, as this was a categorical variable. Multi-level

regression was used to compare sIgA and training load. As the study had multiple testing time points during a week, and to facilitate the examination of between and within player variability, a two-level model was conducted, level 1= training weeks and level 2=players. This was to investigate the variance between weeks and players and the variance within players across those training weeks.

RESULTS

sIgA and URTI

The logistic regression found that as sIgA significantly decreased the risk of contracting an URTI increased ((-0.00537 (0.00268) ug.ml, p=0.046)) (beta (SE)). There was a highly significant decrease of sIgA 1-2 weeks before players contracted an URTI (3.14711 (0.61377) ug.ml, p<0.001)). sIgA was found to decrease by 65% or more 1-2 weeks prior to contraction of an URTI (Table 1). No significant difference (p>0.05) was found between sIgA levels in players with an URTI and players sIgA levels without an URTI (Figure 2), it was only 2-weeks preceding an URTI that players sIgA decreased.

A significant negative association was found between URTI and having a low sIgA value ((-0.00751) 0.00346) ug.ml, p=0.030)). This suggests a player was at more risk of contracting an URTI, with a lower sIgA value. No significant differences in weekly sIgA levels were found over the 10-week period (Figure 3).

Incidences of URTI

Over 10-weeks (70-days testing period; 45-days of training), 15 incidences of URTI were reported out of the 19 players. Of the 15 incidences of URTI, 3 players reported 2 separate

URTI's and the remaining 9 incidences were 9 individual players. These results show that, 21% of players contracted an URTI. The probability of developing an URTI was 0.21; with the odds of contracting an URTI 0.27 and the odds of not contracting an infection were 3.67.

Table 1 insert here

Figure 2 insert here

Secretion and Flow rate and URTI

Secretion rate was found to have a highly significant association with sIgA, indicating that as sIgA increased, secretion rate significantly increased ((0.98804 (0.18565) ug.min-1, $p<0.001$)). No association was found between flow rate and sIgA ((0.00022 (0.00039) ml.min-1, $p>0.05$)). The logistic regression found no significant difference ($p>0.05$) between contracting an URTI and secretion rate ((-0.00106 (0.00137) ug.min-1)) or flow rate ((0.04957 (0.26179) ml.min-1)). Figure 4 shows the weekly variations of flow rate and secretion rate over the course of the 10-week study.

Figure 4 here

sIgA and training load

There were no significant associations ($p>0.05$) found between sIgA and training load (Figure 3) or chronic training load and sIgA ((-0.04038 (0.03498) ug.ml, $p>0.05$)). Additionally, no association was found between players with an URTI and training load.

Training load significantly increased in weeks 2, 6, 7, 8, 9 and 10, which is congruent with the decrease in sIgA levels below baseline levels (Figure 3). However, the decrease in sIgA was not statistically significant. Additionally, it was found that 1-2 weeks prior to the significant decrease of sIgA, leading to an URTI, the players training load increased on average by 49% from the previous week of training. It must be noted that the increase in training load occurred in 13 out of the 15 reported incidences of URTI, the remaining 2 players had a decrease in training load. The players who did not contract an URTI, training load only increased on average by 16% from the previous weeks training.

Figure 3 insert here

DISCUSSION

This is the first study to conduct bi-weekly saliva testing (Monday and Friday morning) in elite Rugby Union players over a 10-week pre-season training period, adopting a stringent method for saliva collection to reduce measurement error.

The main finding of this study was that sIgA may be used as a predictor for likely risk of a subsequent URTI, in elite Rugby Union players. The results indicated that if a player's sIgA decreased by a mean threshold 65% or greater, they were at risk of developing an URTI in the following 2-weeks. These results are similar to Neville et al. (29) with elite professional sailors, and Tsai et al. (42) with elite taekwondo athletes, both finding a significant reduction in sIgA 3-weeks before an URTI occurred. The potential reason for the 2-week decrease in sIgA in the current study and 3-week in previous research, could be the frequency of sample collection. The current study collected saliva bi-weekly, whereas Tsai et al. (42) only collected samples at 7 time points over a 7-week period. Another reason for the differences

between studies could be the different exercise intensities and training volumes (35) and the training environment (i.e. training outdoors compared to indoors, heat, humidity or cold) within which the players train (37).

No significant difference was found between sIgA levels in players with an URTI and players sIgA levels without an URTI. The players' sIgA levels with an URTI returned to the same level as players without an infection. The time taken for a player's sIgA returning to baseline was dependent on the severity of the URTI. Novas et al. (31) also found that tennis players with an URTI had a significant drop in sIgA preceding infection episodes but sIgA did not differ in players with and without an URTI. However, sIgA samples were only collected pre and post 12-weeks, not bi-weekly as was the case in this investigation. Further research has also found no correlation between sIgA levels with an URTI (14, 28). However, due to the infrequent testing of 7 time points over 12-week period (14) and duration of testing, only 3 days (28), a direct comparison cannot be made to the current study. Another reason for the lack of significant differences in sIgA levels in players with and without an URTI, could be due to the fact that coaches and teams' qualified nutritionist were notified of 65% decrease in sIgA, upon which they then put in place a strategy to limit players' further deterioration of the URTI. This may have reduced the risk of additional impingement on training.

A significant association was found between URTI and low sIgA values. This is in agreement with previous studies in both ice hockey players (33) and American football players (9), which found that players with higher incidence of URTI symptoms had significantly lower sIgA levels, compared to players without symptoms of infections. Furthermore, Cunniffe et al. (7) study investigating elite Rugby Union players, also found lower sIgA levels were reported with incidences of URI than those without URI. However, it must be noted, that

saliva samples were only collected monthly (11 occasions) over a whole Rugby season (7), 8 time points over 12-months (9) and 8 times over 5-months (33), rather than weekly. Additionally, only weekly reports of URTI symptoms were recorded in Fahlman et al. (7) and Cunniffe et al. (9), which may have reduced the occurrence date of URTI.

Secretion rate was found to have a significant association with sIgA, as sIgA increased secretion rate significantly increased. Interestingly, no association was found between secretion rate and URTI incidences, even though previous research has found that lower sIgA secretion rate could be used as a better predictor for URTI than sIgA (9, 13). However, it is difficult to compare across studies, due to the different collection methods used and exercise intensities (30). Additionally, saliva measurements have previously been shown to have a large individual variability (29), which was also found in this study with the large standard deviations, this may be the reason why no association was found in the current study. Moreira et al. (26) found as sIgA secretion increased, there was a significant decrease in URTI symptoms during the 2-week detraining period. However, this was with young soccer players (12.9 years old), with only 4 saliva collection points over 12-weeks. In contrast, Novas et al. (31) found no significant differences between sIgA and secretion rate over 12-weeks. However, again infrequent testing of saliva could be the reason for different findings, as saliva collection was only tested pre and post 12-weeks, not bi-weekly as was the case in the current study.

There were no significant associations found between sIgA and training load or chronic load. This contradicts previous research, as Moreira et al. (25) found a significant association between training load and URTI in futsal athletes. As training load decreased, the URTI symptom severity decreased, suggesting higher training periods meant athletes were more

susceptible to developing an URTI. Novas et al. (31) also found higher incidences of URTI were related to higher training loads. The reason for the current study finding no association between sIgA and training load could indicate the appropriate training load management by the coaching staff, ensuring sufficient recovery (31). Interestingly, the findings from the current study showed that 1-2-weeks prior to the decrease of sIgA, which resulted in an infection, the players training load increased by 49%. This is a novel finding and has not been reported in any other study to the authors' knowledge. However, it must be noted that 2 out of the 15 players who contracted an URTI, training load actually decreased. The reason for this may be due to multiple factors that can led to an URTI, including exposures to different bacteria/viral, different immune responses (14) and other sick teammates that train in close proximities (9, 12, 37). These results imply that appropriate prescription and management of training load, along with regular testing of sIgA can help reduce the risk of players developing an URTI. This monitoring, will therefore, help to optimize training and performance, while reducing days missed training.

The study supports the hypothesis that sIgA may be used as a predictor of players at potential risk of contracting an URTI, but in contrast, did not find an association between sIgA and training load. However, it can be seen that the players who contacted an URTI had a large increase in training load prior to significant decrease in sIgA, which led to an URTI. The main strength of this study was the bi-weekly testing and players reporting at any time during a week if they had any URTI symptoms. The 10-week duration, caliber of subjects and comparison to training load (calculated using sRPE x session duration) are further strengths. Saliva sample collection is also non-invasive, easy to collect and time-efficient, meaning it can be used in an applied team setting, causing minimal disruption to the players schedule, unlike blood collection (34).

The results informed practice by being provided to the coaches, who used the results to alter training load and nutrition if they felt it necessary. It must be taken into account that this may have influenced the results, by affecting the overall number of URTI and/or the severity.

A limitation to the study was the collection of only pre-season data. Additionally, only internal training load was calculated using sRPE. Further research needs to be conducted over an entire Rugby season to examine differences in sIgA levels and URTI across pre-season, in-season (competitive season) and the off-season. Additionally, the relationship between sIgA and both internal and external training load data (e.g. GPS) should be examined in future studies of this nature.

URTI are one of the most common illnesses in sport, which can increase the number of training days missed and thereby affect subsequent performance. To help athletes optimize their performance, indicators for the potential risk of contracting an URTI are needed. The findings of the current study suggests that sIgA may be an effective, non-invasive and rapid method of determining if a player is at risk of suffering an URTI.

PRACTICAL APPLICATIONS

The main finding of this study was that a decrease of 65% or more of sIgA meant players were at risk within the following 2-weeks of contracting an URTI. These results provide coaches and support staff with an objective monitoring tool to help reduce the incidences of URTI and missed training days, in an effort to optimize performance. By identifying players at risk of contracting an URTI (i.e. decreased sIgA), coaches can subsequently put a plan in place to reduce the risk of a player contracting an URTI (e.g. reduce training load). It is important results are combined with training load data and evaluated on player-by-player

basis, to appropriately plan and adjust the players training load; meeting the individuals training requirements. Ideally, saliva collection would be bi-weekly, increasing the likelihood of detecting decreased sIgA levels, in order to prevent an URTI occurring. However, weekly testing with stringent method would still be beneficial. Finally, baseline measures should be collected the week prior to pre-season, to gather data that has not been affected by training.

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FIGURES CAPTIONS

Figure 1. Training and match schedule over 10-week pre-season period.

Figure 2. Figure 2. sIgA levels pre, with an URTI and without an URTI. Significant difference between IgA prior to an URTI compared to during and players with no infection. * $p<0.05$

Figure 3. Weekly response over 10-week pre-season of (a) sIgA and (b) Training load compared to week 1 baseline measures. * $p<0.05$ ** $p<0.001$

Figure 4. Weekly averages of secretion and flow rate across 10-weeks pre-season training, compared to baseline. * $p<0.05$ ** $p<0.001$

Table 1. Mean ± SD values for players' pre URTI, with and without an URTI.

FIGURES

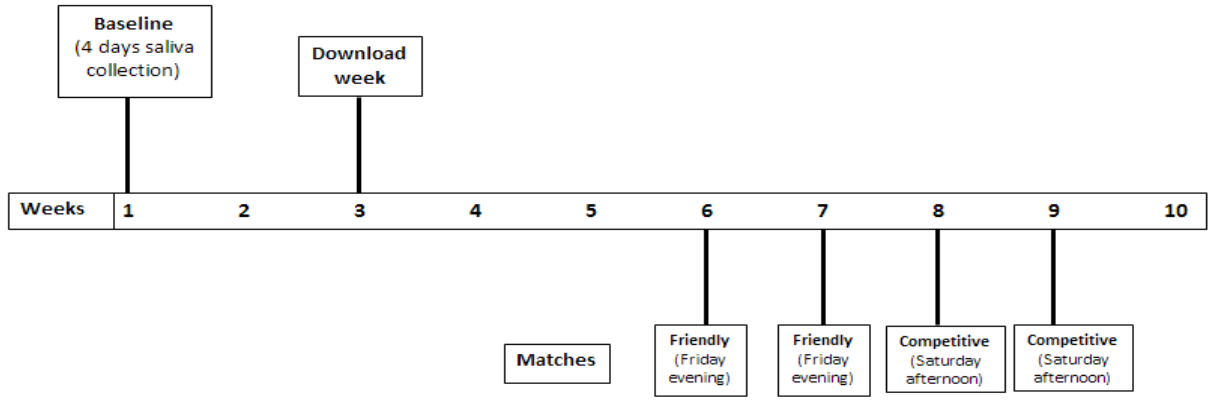


Figure 1. Training and match schedule over 10-week pre-season period

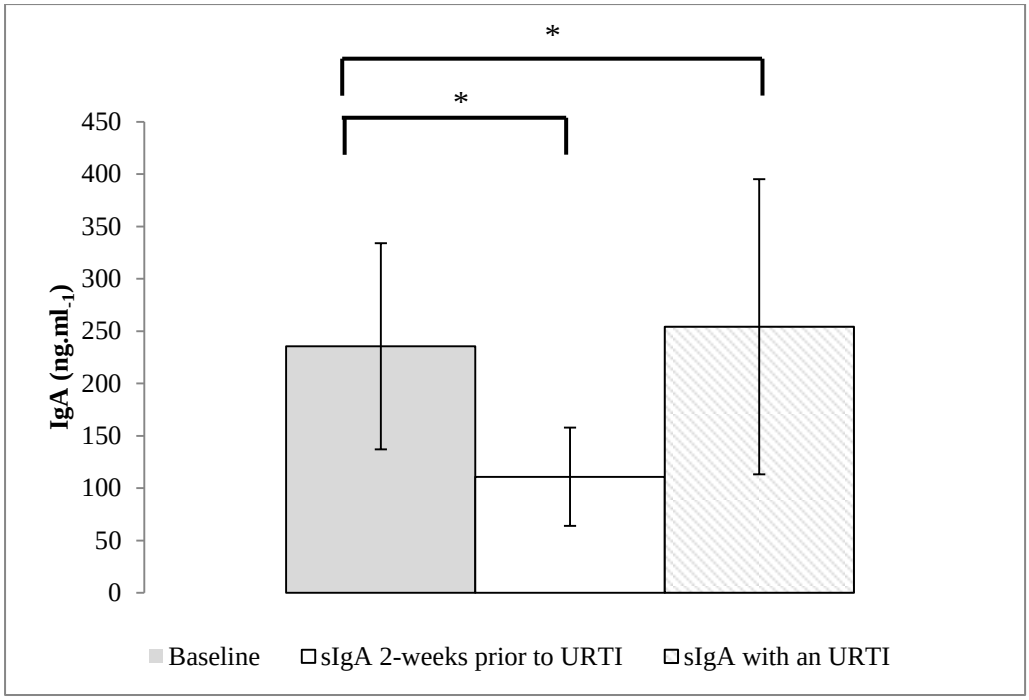
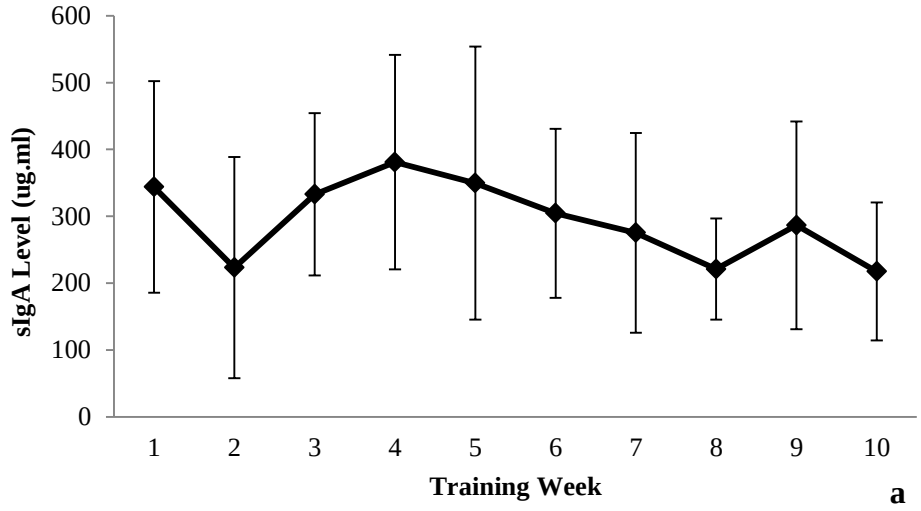


Figure 2. sIgA levels pre, during URTI and with no URTI. Significant difference between IgA prior to an URTI compared to during and players with no infection.

* $p<0.05$



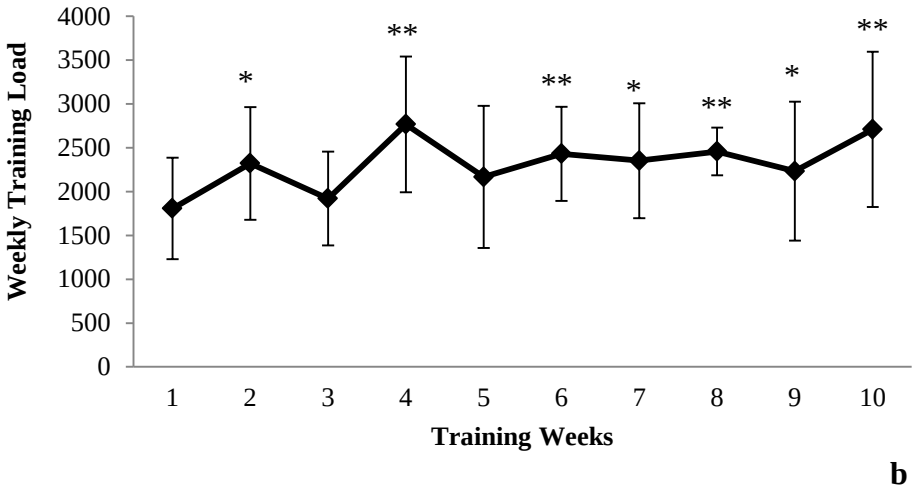


Figure 3. Weekly response over 10-week pre-season of (a) sIgA and (b) Training load compared to week 1 baseline measures.

* $p<0.05$

** $p<0.001$

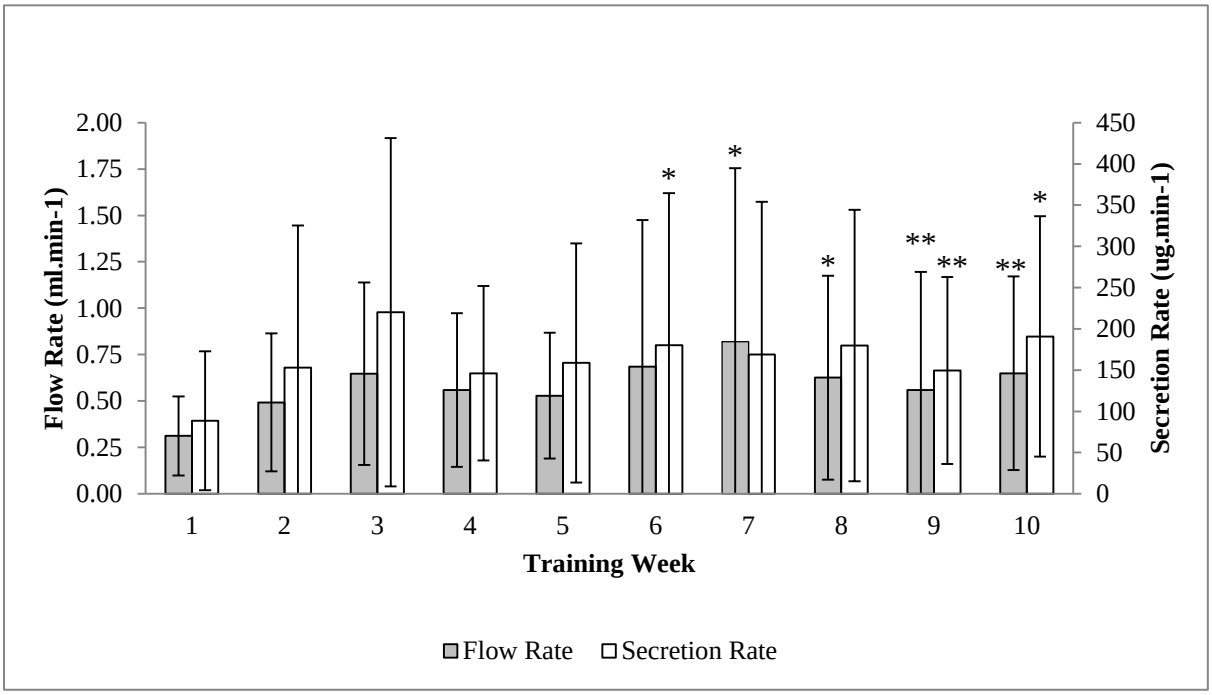


Figure 4. Weekly averages of secretion and flow rate across 10-weeks pre-season training, compared to baseline.

* $p<0.05$

** $p<0.001$

TABLES

Table 1. Mean ± SD values for players' pre URTI, during and without URTI

	sIgA (ug.ml)	Flow Rate (ug.ml-1)	Secretion Rate (ng.ml-1)
All data	257.01 + 152.57	0.59 ± 0.57	163.29 ± 156.10
Players 2-week prior to URTI	110.80 ± 46.89	0.53 ± 0.42	169.94 ± 155.91
Players at the time of URTI	254.22 ± 140.98	0.52 ± 0.42	135.24 ± 107.48
Players without URTI	257.23 ± 153.79	0.60 ± 0.59	164.84 ± 159.76